

Ethological Isolation Between Two Stocks of *Drosophila Adiastola* Hardy¹

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The development of reproductive barriers which prevent free gene exchange between populations is an integral part of the speciation process, at least in most sexually reproducing animals. These "isolating mechanisms" (Dobzhansky, 1937) can be divided into two major classes: premating and postmating isolation barriers. Premating barriers include ecological and ethological differences between populations while postmating barriers include such conditions as hybrid inviability or sterility, or at least some form of reduction in Darwinian fitness in the hybrid individuals (see reviews in Mayr, 1970 and Dobzhansky, 1970).

It has been proposed that genetic divergence, which arises as a by-product of adaptational shifts (natural selection) to different environmental conditions, provides the basis for postmating isolation. However, there are two contrasting views which seek to explain the derivation of premating isolation mechanisms, especially behavioral isolation. It has been postulated that ethological isolation evolves as an "ad-hoc" product of natural selection against those hybrid individuals which have reduced fitness in comparison to either parental populations (Fisher, 1930 and Dobzhansky, 1940). In contrast, Muller (1942) suggested that premating as well as postmating barriers arise as by-products of genetic divergence prior to the attainment of sympatry.

Preliminary hybridization experiments between two laboratory stocks of *Drosophila adiastola* Hardy indicate that behavioral differences exist despite the apparent fertility of the F₁ progeny. As both strains were derived from the same naturally interbreeding population, the premating behavioral differences appear to have been established at or since the time the laboratory strains were isolated.

MATERIALS AND METHODS

The two stocks of *D. adiastola* used in this study were each started from single wild females both collected from Kaulalewelewe, West Maui, Hawaii at approximately 3000 feet elevation. The M55G17 stock, established from progeny of a female collected by H. L. Carson on July 15-16, 1969, was involved in a number of "crashes" in population size during its six years in the laboratory. The precise number of these "bottlenecks" and the condition of the stock during these situations were not recorded. The T79B3 stock was established from progeny of a female collected by K. Y. Kaneshiro on September 15-16, 1975. Thus, the only apparent differences between the two stocks were the length of time that each has been maintained in the laboratory and the population crashes which the M55G17 stock had undergone. Because of these population crashes it appeared that the M55G17 stock had encountered severe genetic drift situations at least four or five times during its existence in the laboratory. The T79B3 stock, on the other hand, has been maintained at a relatively large size and no depression of population size has occurred.

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Virgin females were collected daily and allowed to mature for one week before use in male-choice experiments. Males were also collected daily but allowed to mature for two weeks before use in male-choice experiments. Maturation, in this study, was defined as the age at which females would accept the courtship overtures of males, and the age at which males could inseminate females. The maturation periods for both sexes were determined on the basis of previous observations (Arita, unpublished data). Females were apparently ready to accept males at two days after eclosion from the puparium. By seven days after eclosion, most of the females were sexually mature and readily accepted the courtship overtures of mature males. Males required eight to nine days after eclosion before motile sperm were observed in the testes. At 10 to 11 days after eclosion, most of the males were capable of inseminating mature females. Thus a period of seven days for females and 14 days for males was considered as adequate maturation time for the two sexes.

Since the individuals of these two stocks were not distinguishable morphologically, females had to be marked in some way to enable us to readily distinguish between them. This was accomplished by immobilizing the females by exposure to 0° C temperature for approximately 15 minutes and placing a small drop of Testor's quick drying enamel paint on the mesonotum just anterior to the scutellum. It was shown by Ohta (1977) that this technique of marking Hawaiian *Drosophila* species had little or no effect on the results of behavioral experiments. Such was also found to be the case with *D. adiastola*.

After marking the females and maintaining the sexes separate for the duration of the maturation period, the flies were used in male-choice experiments as described by Ahearn et al. (1974) and Kaneshiro (1976). Two females, one from each of the two stocks, were placed in a culture vial with a single male which was from the same stock as one of the two females. Both females used in each replicate were of the same age. The number of homogamic versus the number of heterogamic matings were obtained in one of two ways. First, direct observations of copulating pairs were recorded and second, if no copulation was observed in a vial after six to seven days of periodic observation (usually between 8:00-11:00 a.m.), both females were dissected to determine insemination by the presence or absence of sperm in the spermatheca or any other part of the reproductive tract.

Although Ahearn et al. (1974) used the total number of homogamic and heterogamic matings in their calculation of isolation, Kaneshiro (1976) argued that only vials in which one of the females was inseminated should be used in the calculation. He pointed out that a female which has been inseminated becomes aggressively non-receptive to the courtship overtures of the male and therefore the insemination of the second female in the vial essentially becomes a "no-choice" situation.

The degree of isolation is measured by calculation of the Charles-Stalker Index of Isolation (Stalker, 1942) which is the frequency of homogamic matings minus the frequency of heterogamic matings divided by the sum of these frequencies. In our experiments, as in those of Kaneshiro (1976), the sum of the frequencies of homogamic and heterogamic matings always equaled 1.0 since only trials in which at least one but only one of the females is inseminated were counted. The number of replicates which had both females inseminated and those in which both females were virgins were recorded (Table 1) but were not used in the analysis of isolation. A statistical test of significance was calculated for each reciprocal combination by applying the formula for the test of proportions, $C = (\hat{p} - p) / \sqrt{pq/n}$, where \hat{p} , the

mean of the sampling distribution, is the unbiased estimate of p (the mean of a normal distribution = 0.5); and pq/n is the variance of the sampling distribution. Thus, $C = (\hat{p} - 0.5) / \sqrt{.25/n} = 2\sqrt{n}(p - 0.5)$. At the 5% confidence interval, the null hypothesis that mating between the two stocks is random is accepted if $-1.96 < C < +1.96$.

RESULTS AND DISCUSSION

The results of the male-choice experiments are reported in Table 1. Clearly, females of T79B3 strongly discriminated against males of M55G17. However, males of T79B3 were accepted randomly by females of both M55G17 and T79B3. The asymmetrical isolation observed between these two stocks of *D. adiastola* is similar to the situation reported by Kaneshiro (1976). He found that pair-wise comparisons of allopatric species in the *planitibia* complex of Hawaiian *Drosophila* showed, for the most part, striking asymmetry in isolation indices between reciprocal combinations. To explain the significance of such a phenomenon (the asymmetrical isolation), Kaneshiro presented the following hypothesis:

"The founder principle is hypothesized to play a major role in the interisland speciation of Hawaiian *Drosophila* (Carson 1968, 1971). Speculatively, the mechanics of this evolutionary process may provide the basis for justification of the above arguments. A founder individual (single fertilized female) represents only a portion of the total gene pool of the ancestral population. The courtship pattern of the derived species therefore has elements in common with its ancestral population; but on the other hand, a few elements of the total courtship pattern of the ancestral population is changed ('lost') by the 'genetic revolution' which accompanies the founder event in the derived population. In this way, females of the derived species may recognize and accept the courtship overtures of males of the ancestral species since these males contain all the courtship elements present in conspecific males. However, females of ancestral species show strong discrimination against males of derived species since these males contain only a portion of the total courtship pattern of conspecific males."

The same argument can be used to explain the phenomenon observed in this study. The founder-flush theory of speciation proposed by Carson (1968, 1971) essentially reflects an accelerated form of genetic drift. As discussed in the "Materials" section of this paper, the M55G17 stock which has been maintained in the laboratory for over six years has been subjected to numerous, although unintentional, bottlenecks. Relatively severe genetic drift has probably accompanied these periods when the stock was reduced to a small number of individuals. If Kaneshiro's (1976) theory is correct, males and females of M55G17 may have lost the genetic basis for certain elements from their courtship pattern. Individuals from T79B3, on the other hand, have a courtship pattern which is probably similar to the ancestral condition since it has not gone through any kind of bottleneck event, except for the F_1 generation from the original female from which the stock was derived. Thus, females of M55G17 will readily accept males of T79B3 since these females require fewer elements than are present in the courtship repertoire of T79B3 males. Conversely, females of T79B3 only occasionally accept M55G17 males since these males have lost elements required by the T79B3 females.

CONCLUSIONS

The data reported here, as well as those presented by Kaneshiro (1976), appear to support Muller's model for the origin of premating ethological isolating mechanisms. We suggest that the severe genetic drift conditions, which in all probability perturbed the genetic composition of the M55G17 stock, played an important role in promoting behavioral changes in this stock. At least, natural selection for premating isolating mechanisms through the selection against hybridization was not responsible for the behavioral isolation observed between these two stocks of *D. adiaestola*. Preliminary observations indicate that hybrid individuals obtained from crosses between the two stocks are fully fertile at least in the F₁ generation (Arita, unpublished data). Thus, it would appear that premating barriers have evolved in the absence of postmating barriers.

TABLE 1. *Mating tests between two stocks of Drosophila adiaestola* Hardy.
M = M55G17, T = T79B3.

♀	X	♂	n	Vials with no matings	Vials with two matings	Homogamic matings only	Heterogamic matings only	I ¹	C ²
M		M,T	128	68	12	39	9	0.62	4.33
T		M,T	83	18	15	24	26	-0.04	-0.29

¹I = Charles-Stalker Isolation Index.

²The null hypothesis that mating is random is accepted when $-1.96 < C < +1.96$.

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